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Recombinant DNA Research; Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of Actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the November 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules (45 FR 77384).

EFFECTIVE DATE: July 1, 1981

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205 (301) 496–6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several major actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These proposed actions were published for comment in the **Federal Register** of March 20, 1981, and reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on April 23–24, 1981. In accordance with Section IV–E–1–b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment.

Following this announcement, there appears in a separate section of the **Federal Register** the revised NIH Guidelines for Research Involving Recombinant DNA Molecules. These revised Guidelines differ from the version of the Guidelines promulgated on November 21, 1980 (45 FR 77384) by incorporating within them both the changes in the Guidelines which were recommended at the RAC meeting of January 8–9, 1981, and promulgated on March 12, 1981 (46 FR 16452), and the changes in the Guidelines which were recommended at the RAC meeting of April 23–24, 1981, and which are discussed in this announcement.

I. Revision of Guidelines for Recombinant DNA Experiments Involving Escherichia Coli K–12 and Saccharomyces Cervisiae Host-Vector Systems

Containment requirements and administrative procedures for the conduct of most recombinant DNA experiments involving EKl *Escherichia coli* strain k–12 and laboratory strain *Saccharomyces cerevisiae* host-vector systems have been specified in Section III–O of the Guidelines. The chairpersons of Institutional Biosafety Committees, at a meeting in Washington, D.C. on November 24–25, 1980, passed by a large majority a resolution that experiments currently covered by Section III–O should be made exempt from the Guidelines. The chairpersons felt that the risks associated with these experiments are negligible, and that exemption of experiments currently covered by Section III–O would reduce paperwork by approximately 90%.

This proposal was discussed by the RAC at its meeting on January 8–9, 1981. In "straw votes" at the meeting, an overwhelming majority of RAC members favored doing something to reduce materially or eliminate the paperwork and reporting functions for experiments covered by Section III–0; about half the RAC members felt serious consideration should be given to exempting entirely from the Guidelines experiments currently covered by Section III–0. The RAC requested that a series of options be developed and published for comment in the **Federal Register.** Accordingly, a series of options was published for comment in the **Federal Register** of March 20, 1981 (46 FR 17994), and considered at the April 23–24, 1981 meeting of the RAC.

Three major options were published for comment. Option A would have allowed review by an institutional official rather than requiring review by the IBC of experiments currently covered by Section III–0. Option B would have eliminated the registration and review requirements for experiments currently covered under Section III–0. Option C would have exempted from the Guidelines experiments currently covered by Section III–0. Under each of these three major options a series of suboptions was proposed for alternative ways of treating certain types of experiments which were already being treated differently from the majority of experiments under Section III–0. These are experiments involving the cloning in *E. coli* K–12 of DNA from Class 3 CDC agents, and experiments involving a deliberate attempt to have *E. coli* K–12 efficiently express a eukaryotic gene. Under Option C, additional suboptions were proposed regarding biological and physical containment, and large-scale experiments (i.e., greater than 10 liters of culture).

During the 30 day comment period, only one commentator responded referring to the proposed changes as "* * * an attempt to bring the Guidelines into a state of consistency with present evaluations of the potential for hazard in recombinant DNA experiments. As I see that evaluation, there is widespread agreement that experiments involving *E. coli* K–12 or *S. cerevisiae* host-vector systems will not generate harmful organisms. * * *" One commentator, who wrote after the comment period, was opposed to the proposed changes "until adequate risk assessment studies have been performed." The RAC discussed the proposed changes at its meeting on April 23. Dr. Edward Adelberg, Chairman, Yale University Institutional Biosafety Committee, summarized the concerns of the chairpersons of IBCs at their meeting in November 1980. He said that the chairpersons voted heavily in favor of an exemption for these experiments, but noted that any one of the three proposed options would greatly reduce what was considered unnecessary paperwork. The RAC then discussed in detail the various options and suboptions.

After a series of motions and amendments, the RAC by a vote of 13 in favor, 8 opposed, with no abstentions, passed Option C with Suboptions C–1–b, C–2–a, C–3–a, C–4–b as modified at the meeting, and C–5–b. Under the recommendation passed by the RAC, experiments currently covered by Section III–0 would be removed from Section III–0. These experiments would be exempted under Section I–E–5. The exemption would apply to all nonprohibited experiments which use either *E. coli* K–12 or laboratory strains of *Saccharomyces cerevisiae* as the host-vector system with the following provisos:

- The exemption would be qualified with the statement that "(a) the *E. coli* host shall not contain conjugation proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (49) shall be used as vectors." This qualification would then be qualified by the statement that experiments "involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information (35) with *E. coli* may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages."
- Language would be added to the Guidelines to explicitly make

Prohibition I–D–6 concerning large-scale applicable to these experiments.

- The entry in Appendix C which would effect the exemption under Section I–E–5 would contain a statement that although exempt, for these experiments "Pl physical containment conditions are recommended."
- No prior review of experiments involving attempts at deliberate expression of a eukaryotic gene in *E. coli* K–12 would be required.
- Experiments involving the DNA of CDC Class 3 agents would not be exempted. Containment might be specified at P1, P2 or P3, and review by the IBC would be required. If there is any chance that the original Class 3 agent can be regenerated from the cloned DNA, the containment level shall be no lower than that appropriate for the agent itself.

I accept these recommendations. The last recommendation dealing with Class 3 agents is unclear as to the appropriate physical containment level, and as to how this level is to be established. To clarify this, the following language regarding DNA from CDC Class 3 agents in *E. coli* K–12 is being added to Appendix C:

"Experiments using DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment. Lower containment levels may be specified by NIH (See Section IV–E–1–b–(2)–(e)). Expermiments in this category require prior IBC review and approval."

These decisions regarding *E. coli* K–12 and *Saccharomyces cerevisiae* host-vector systems are being promulgated under Exemption I–E–5, and appear in Appendix C of the revised Guidelines.

Although the movement of experiments currently under Section III–0 to the exempt category leaves no entries in Section III–0, I am retaining a Section III–0 in the Guidelines. In the future, experiments may be placed under Section III–0 by their listing in Appendix H to the Guidelines. These experiments may be performed at P1 physical containment, and IBC review prior to initiation of the experiment will not be required.

II. Containment Levels for Recombinant DNA Experiments Involving Bacillus Subtilis

Dr. Donald Dean of the Ohio State University requested that the status of recombinant DNA experiments involving *Bacillus subtilis* as host-vector systems be reevaluated. Currently, two asporogenic *B. subtilis* strains, RUB331 and BGSC 1S53 have been certified, with the plasmids pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124, as HV1 host-vector systems.

The asporogenic mutant derivative of *B. subtilis*, ASB298, has been certified with the above plasmids as HV2 host-vector systems.

Dr. Dean proposed that any *Bacillus subtilis* strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can be used for cloning DNA from any nonprohibited source, using vectors indigenous to *B. subtilis*, under the same conditions specified by the RAC for *E. coli* K–12 and *Saccharomyces cerevisiae* host-vector systems.

Dr. Dean further proposed that the following items be added to Appendix E:

"*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used with vectors indigenous to *B. subtilis* for the cloning of DNA from any CDC Class 1 organism under P2 conditions."

"*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used with vectors indigenous to *B. subtilis* under P1 conditions for the cloning of DNA from any Class 1 *Bacillus* species."

In support of his proposal, Dr. Dean noted that *B. subtilis* is not a pathogen and that the organism is well characterized, and a great deal is known of its genetics.

An announcement of Dr. Dean's proposal appeared in the **Federal Register** on March 20, 1981 (46 FR 17996). During the thirty day comment period seven letters supporting the proposal were received. One of the letters cited data showing that (1) *Bacillus subtilis* spores either cannot germinate, or upon germination in the ileum or colin die very quickly, and (2) the viable count of *B. subtilis* in soil or water decreases much more rapidly than that of *E. coli*.

The RAC evaluated Dr. Dean's request at the April 23–24, 1981, meeting. Noting that the **Federal Register** language would permit use of "indigenous vectors," the RAC questioned the advisability of permitting use of all *Bacillus* vectors, and recommended excluding from use as vectors any that have as hosts the pathogens, *Bacillus cereus* or *Bacillus anthracis*.

By a vote of 12 in favor, 0 opposed, with 5 abstentions, the RAC voted to recommend that any *Bacillus subtilis* strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can be used for cloning DNA from any nonprohibited source at the same containment conditions as used for *E. coli* K–12 and *Saccharomyces cerevisiae* host-vector systems, using indigenous plasmid and phage vectors whose host range does not include *Bacillus anthracis* or *Bacillus cereus*.

I accept this recommendation, and this decision is being promulgated under Exemption I–E–5, and appears in Appendix C of the revised Guidelines. Information about *Bacillus subtilis* HV1 and HV2 systems has been accordingly removed from Appendix D and placed in Appendix F.

By the same vote, the RAC recommended approval of Dr. Dean's additional two specific proposals concerning *Bacillus subtilis* host-vector systems, with the limitation that *Bacillus* plasmids or phages whose host range includes *Bacillus cereus* or *Bacillus anthracis* may not be used as vectors.

I accept these recommendations and these decisions are promulgated as new entries in Appendix E of the revised Guidelines.

III. Streptomyces HV1 Host-Vector Systems

Dr. Stanley Cohen of Stanford University requested that "*Streptomyces coelicolor* and the related organisms with which *S. coelicolor* naturally exchanges genetic information (e.g., *S. lividans*, *S. parvulus*, and *S. griseus*), using *Streptomyces* plasmids SPC2, SLP1.2, pIJ101, actinophage phiC31, and their derivatives as vectors, be approved as HV1 host-vector systems."

During the 30 day comment period, no comments were received on this proposal.

The RAC reviewed this request at the April 23–24, 1981, meeting, and noted that these organisms are not pathogenic. There has been extensive experience with large-scale culture of these organisms with no known hazards.

By a vote of 10 in favor, 0 opposed, with 8 abstentions, the RAC recommended approval of this proposal. I have reviewed this recommendation, and I am certifying the four specific *Streptomyces* species cited in the proposal as HV1 host-vector systems. These host-vector systems have been added to Appendix D of the Guidelines.

IV. Guidelines for Recombinant DNA Experiments with Genes Coding for Toxins

The RAC, at its September 25–26, 1980, meeting, requested that an *ad hoc* working group be formed to evaluate Section I–D–2 of the Guidelines. Section I–D–2 prohibits the "deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates (2A) (e.g., botulimum or diphtheria toxins; venoms

from insects, snakes etc.)." an *Ad hoc* Working Group on Toxins composed of Drs. Werner Maas and Alan Bernheimer of New York University, Dr. John Collier of Yale University, Dr. Michael Gill of Tufts University, Dr. Susan Gottesman of NIH, Dr. Myron Levine of the University of Maryland, and Dr. James Mason of the Utah State Department of Health, was convened to recommend containment conditions for recombinant DNA experiments with genes coding for toxins. The group conversed by telephone on November 4 and November 21, 1980, was convened at the National Institutes of Health on January 7, 1981, and participated in telephone conference calls on February 23 and March 2, 1981. The following proposal, developed by the *ad hoc* group, appeared in the **Federal Register** of March 20, 1981 (46 FR 17996):

"A. Section I–D–2 of Section I–D, Prohibitions, would be amended to read as follows:

"I–D–2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, *Shigella dysenteriae* neurotoxin). Guidelines for the cloning of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight are specified in Appendix G.

B. A new Appendix G, would be added to the Guidelines as follows:

Appendix G—Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Toxins for Vertebrates

1. General Information

"Appendix G specifies the containment to be used for the cloning of genes coding for the biosynthesis of toxins for vertebrates. Cloning of genes coding for toxins for vertebrates that have an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, *Shigella dysenteriae* neurotoxin) is prohibited. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. A list of toxins classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxins not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with the *ad hoc* working group on toxins prior to inclusion of the toxin on the list. (See Section IV–E–1–b–(3)–(i)).

2. Containment Conditions for Cloning of Toxin Genes in E. coli K–12

(a) Cloning of genes coding for toxins for vertebrates that have an LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight (e.g., diphtheria toxin, abrin, *Clostridium perfringens* epsilon toxin) may proceed under P2+EK2 or P3+EK1 containment conditions.

(b) Cloning of genes for the biosynthesis of toxins for vertebrates with an LD_{50} in the range of 1 microgram to 100 micrograms per kilogram body weight may proceed under Section III–O (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordatella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

(c) Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins, whose effects are confined to the stimulation of intestinal secretion and whose effects can be entirely reversed by administration of electrolyte solutions, shall be subject to Section III–O. These are cholera toxin, the heat labile toxins of *E. coli, Klebsiella,* and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *E. coli* and of *Yersinia enterocolitica.*

3. Containment Conditions for Cloning of Toxins Genes in Organisms Other Than E. coli K–12

"Requests involving the cloning of genes coding for toxins for vertebrates in host-vector systems other than *E. coli* K–12 will be evaluated by ORDA, which will consult with the *ad hoc* working group on toxins. (See Section IV–E–1–b–(3)–(j)).

C. Section V, Footnote 2A would be modified to delete the words: 'toxins potent for vertebrates (Section I–D–2).'

D. A new Section IV–E–1–b–(3)–(i) would be added as follows: 'IV–E–1–b–(3)–(i). Adding new entries to the list of toxins for vertebrates. (See Appendix G.)'

E. A new Section IV–E–1–b–(3)–(j) would be added as follows: 'IV–E–1–b(3)–(j). Approving the cloning of toxins genes in host-vector systems other than *E. coli* K–12. (See Appendix G.)'"

During the thirty day comment period, no comments were received. However, two letters were received after the thirty day comment period. One expressed concern about experiments involving genes which control bacterial toxins recommended "against relaxation of the Guidelines." The other urged the proposed changes not be made "until adequate risk assessment studies have been performed."

Several members of the *ad hoc* Working Group on Toxins, who were members of the RAC, presented the proposal to the RAC at the April 23–24, 1981, meeting. Working group members characterized the proposal as conservative.

Noting the RAC recommendation at this meeting concerning containment conditions for *E. coli* K–12 and *Saccharomyces cerevisiae* host-vector systems currently covered under Section III–O of the Guidelines, *ad hoc* Working Group members recommended that language in the proposed Appendix G be amended. They said the original intent of the *ad hoc* group with regard to cloning genes for toxins with an LD_{50} in the range of 1 to 100 micrograms, as well as for cloning enterotoxin genes, was to specify P1+EK1 containment. Thy felt it would be inappropriate to "exempt" from the Guidelines the cloning of toxin genes at this time. In response, the RAC recommended that Section 2–(b) and 2–(c) in proposed Appendix G be amended by substituting "P1+EK1" for "Section III–O."

RAC also discussed the appropriateness of the introductory language ". . . whose effects are confined to the stimulation of intestinal secretion and whose effects can be entirely reversed by administration of electrolyte solutions" in Section 2–(c) of proposed Appendix G, and recommended its deletion.

RAC also discussed the intention of the working group that Appendix G specifications override other specifications of the Guidelines (e.g., exemptions or return to host of origin experiments). RAC recommended this conservative approach at this time, and specified wording to be added at the end of Section I–D–2 indicating this.

Finally, RAC indicated a desire that the NIH be kept abreast of experiments dealing with the cloning of toxin genes, and amended the proposed language to require prior registration with ORDA of experiments involving the cloning of toxin genes.

After incorporating these changes, the RAC by a vote of 18 in favor, 0 opposed, with 1 abstention, recommended the following amended language:

A. Section I–D–2 of Section I–D, Prohibitions, would be amended to read as follows:

I–D–2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, *Shigella dysenteriae* neurotoxin). Guidelines for the cloning of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight are specified in Appendix G, which overrides other parts of the Guidelines (e.g., exemptions, return to host of origin, etc.).

B. A new Appendix G, would read as follows:

Appendix G—Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Toxins for Vertebrates

1. General Information

Appendix G specifies the containment to be used for the cloning of genes coding for the biosynthesis of toxins for vertebrates. Cloning of genes coding for toxins for vertebrates that have an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, *Shigella dysenteriae* neurotoxin) is prohibited. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. Experiments involving genes coding for toxins with an LD_{50} of 100 micrograms or less per kilogram body weight shall be registered with ORDA prior to initiating the experiments. A list of toxins classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxins not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with the *ad hoc* Working Group on toxins prior to inclusion of the toxin on the list. (See Section IV–E–1–b–(3)–(i)).

2. Containment Conditions for Cloning of Toxin Genes in E. coli K–12

(a) Cloning of genes coding for toxins for vertebrates that have an LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight (e.g., diphtheria toxin, adbrin, *Clostridium perfringens* epsilon toxin) may proceed under P2+EK2 or P3+EK1 containment conditions.

(b) Cloning of genes for the biosynthesis of toxins for vertebrates with an LD_{50} in the range of 1 microgram to 100 micrograms per kilogram body weight may proceed under P1+EK1 containment conditions (e.g., *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas Aeruginosa* exotoxin A, *Bordatella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

(c) Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to P1+EK1 containment conditions: cholera toxin, the heat labile toxins of *E. coli, Klebsiella,* and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of *E. coli* and of *Yersinia enterocolitica.*

3. Containment Conditions for Cloning of Toxins Genes in Organisms Other Than E. coli K–12

Requests involving the cloning of genes coding for toxins for vertebrates in host-vector systems other than *E. coli* K–12 will be evaluated by ORDA, which will consult with the *ad hoc* working group on toxins. (See Section IV–E–1–b–(3)–(i)).

C. Section V, Footnote 2A would be modified to delete the words: 'toxins potent for vertebrates' (Section I–D–2).

D. A new Section IV–E–1–b–(3)–(i) would be added as follows:

IV–E–1–b–(3)–(i). Adding new entries to the list of toxins for vertebrates. (See Appendix G.)

E. A new Section IV–E–1–b–(3)–(j) would be added as follows:

IV–E–1–b–(3)–(j). Approving the cloning of toxin genes in host-vector systems other than *E. coli* K–12. (See Appendix G.)

I accept these recommendations with additional modifications as stated below.

A further issue concerning cloning of toxin genes was raised by the *ad hoc* Working Group and discussed by RAC during the evaluation of a proposal from Dr. John Murphy of Harvard University, i.e., appropriate containment for cloning the diphtheria toxin gene in *E. coli* K–12 (See below, Part VI of this document). The new Section I–D–2 and Appendix G of the Guidelines uses a cutoff of LD_{50} of 100 nanograms per kilogram of body weight to separate those toxins the cloning of whose genes is prohibited under Section I–D–2 from those allowed to be cloned at P2+EK2 or P3+EK1.

Diphtheria toxin falls very close to this cut-off line. Pharmacological toxicity data for diphtheria toxin demonstrates that the LD_{50} for the most sensitive animal tested, the guinea pig, is 160 nanograms per kilogram body weight. The LD_{50} in humans is estimated to be equal to or less than 100 nanograms per kilogram body weight. This figure was extrapolated from an incident in Japan in which children were inadvertently injected with diphtheria toxin rather than diphtheria toxoid. After much discussion, RAC recommended that experiments by Dr. John Murphy involving the cloning of the gene for the biosynthesis of diphtheria toxin be conducted under P4 physical containment. The RAC realized that this recommendation results in an inconsistency between the recommendation in Appendix G concerning cloning of diphtheria toxin and the recommendation concerning Dr. Murphy's proposal. They noted that cloning the gene for diphtheria toxin could be moved to the prohibited category in general, and Dr. Murphy's proposal could be considered an exemption to the prohibition. Based upon the RAC recommendation and the data available concerning the pharmacological toxicity of diphtheria toxin, I am removing diphtheria toxin from category 2–(a) of Appendix G, "genes coding for toxins that have an LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight," and placing it under section I–D–2.

Accordingly, revised Section I–D–2 will read as follows:

I–D–2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin). Guidelines for the cloning of DNAs containing genes coding for the biosynthesis of toxins which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight are specified in Appendix G, which overrides other parts of the Guidelines (e.g., exemptions, return to host of origin, etc.).

Section 2–(a) of Appendix G will read as follows:

(a) Cloning of genes coding for toxins for vertebrates that have an LD_{50} in the range of 100 nanograms to 1000 nanograms per kilogram body weight (e.g., abrin, *Clostridium perfringens* epsilon toxin) may proceed under P2+EK2 or P3+EK1 containment conditions.

With these modifications, I accept the RAC recommendations concerning the cloning of genes for the biosynthesis of toxins for vertebrates.

Experiments currently underway in *E. coli* K–12 host-vector systems involving genes coding for toxins with an LD_{50} of 100 micrograms or less per kilogram of body weight should be registered with ORDA within 90 days.

It is recognized that the requirements of revised Section I–D–2 and new Appendix G may result in more stringent standards for the cloning of toxin genes than interpretations under the previous Guidelines. Appendix G allows the cloning of toxin genes in *E. coli* K–12 only, and overrides other parts of the Guidelines. Under interpretations of the previous Guidelines, for example, investigators may be conducting recombinant DNA experiments with toxins not listed in Appendix G, or conducting experiments at lower containment levels than now specified in Appendix G, or using host-vector systems other than *E. coli.* K–12 as in "self cloning" experiments. Investigators currently conducting experiments that do not meet the requirements of new Appendix G and that have not already been acted upon individually by NIH must contact ORDA for a case-by-case review within 90 days. However, investigators currently conducting such experiments may continue with their ongoing projects until these projects have been evaluated by NIH.

V. Request for Permission To Clone the Vibrio Cholerae Enterotoxin Gene in E. Coli K–12

Dr. J. J. Mekalanos of Harvard Medical School requested permission to clone in *E. coli* K–12 the *Vibrio cholerae* enterotoxin gene. In support of his request, Dr. Mekalanos noted that *E. coli* elaborates a heat-labile enterotoxin (LT), which has been shown to share a high degree of structural, antigenic and DNA sequence homology with cholera toxin.

Dr. Mekalanos proposes to perform the investigation in three stages; i.e., (1) the cloning in *E. coli* K–12 of DNA restriction fragments of *V. cholerae* known to contain sequences homologous to the A subunit of the LT gene; (2) the cloning in *E. coli* K–12 of DNA restriction fragments of *V. cholerae* containing sequences homologous to the B subunit of the LT gene, and (3) the cloning in *E. coli* K–12 of restriction fragments of *V. cholerae* containing sequences homologous to both the LT A and B subunit genes.

Dr. Mekalanos' request appeared in the **Federal Register** of March 20, 1981 (46 FR 17997). During the thirty day comment period, no comments were received.

RAC discussed Dr. Mekalanos' request at the April 23–24, 1981 meeting. RAC reviewers indicated that P1+EKl containment would be adequate for all three stages of the proposed experiments. By a vote of 15 in favor, 0 opposed, with 1 abstention, the RAC recommended approval of the proposal at P1+EKl containment.

This recommendation is in agreement with part 2-a of Appendix G dicussed in the previous item of this decision document and is permitted under that part.

VI. Cloning and Expression of DNA Coding for Diphtheria Toxin

Dr. John Murphy of Harvard University, in a letter dated March 11, 1981, proposed to clone in *E. coli* K–12, the 3.9 kb Bam restriction fragment and/or the 8.6 kb Eco fragment of *Corynephage Beta* carrying the stuctural gene for diphtheria toxin.

Dr. Murphy advanced the following arguments in support of his proposal:

(1) Studies of diphtheria toxin-producing organisms would yield valuable data for risk assessment;

(2) The localization of diphtheria toxin in *E. coli* would be determined as well as whether the toxin might be secreted by *E. coli;*

(3) Mutagenesis of the tox gene would be used to elucidate (a) diphtheria toxin interaction with the eukaryotic cell toxin receptor, and (b) the mechanism of fragment A translocation into the eukaryotic cell cytosol.

Dr. Murphy proposed to perform the experiments under P4 conditions at the Frederick Cancer Research Center or at the NIH if the RAC recommended the P4 level of containment.

A **Federal Register** announcement of Dr. Murphy's requet appeared on March 20, 1981 (46 FR 17997). During the thirty day comment period, no comments were received.

The RAC discussed the proposal at the April 23–24, 1981 meeting. Members of the *ad hoc* Working Group on Toxins presented to the RAC the data gathered by the group on diphtheria toxin. The data showed that the LD_{50} in the most sensitive animal tested, the guinea pig, was 160 nanograms per kilogram body weight. The LD_{50} in humans is estimated to be equal to or less than 100 nanograms per kilogram body weight. This figure was estrapolated from an incident in Japan in which children were inadvertently injected with diphtheria toxin rather than diphtheria toxoid. By a vote of 15 in favor, 0 opposed, with 1 abstention, the RAC recommended approval of Dr. Murphy's proposal at P4 containment.

I accept this recommendation, and the following entry, number 30, is added to Appendix E:

> Permission is granted to clone in *E. coli* K–12 under P4 containment conditions, restriction fragments of *Corynephage Beta* carrying the structural gene for diphtheria toxin.

VII. Proposals Involving the Expression of Foot and Mouth Disease Viral Coat Proteins in Saccharomyces Cerevisiae, Bacillus Subtilis, and Tissue Culture Systems

The RAC at its April 23–24, 1981 meeting reviewed proposed experiments jointly submitted by Genentech, Inc., South San Francisco, California, and the United States Department of Agriculture, Plum Island Animal Disease Center, Greenport, New York, to study the expression of Foot and Mouth Disease Virus (FMDV) sequences in *Bacillus subtilis, Saccharomyces cerevisiae,* and mammalian tissue culture systems, with the goal of production of a viral subunit vaccine for Foot and Mouth Disease. This proposal was an extension of their prior NIH approved proposal for the study of the expression of the capsid protein of FMDV in *E. coli* K–12.

A summary of the request was published for comment in the **Federal Register** of March 20, 1981 (46 FR 17996). During the 30 day comment period, no comments were received on the proposals.

The RAC recommended that the work be permitted at P1 physical containment with both the *B. subtilis* and *S. cerevisiae* aproved host-vector systems if the subgenomic FMD segments were restricted to those sequences which map between 500 and 4100 of the FMD viral genome. Separate motions to approve these experiments at P1 physical containment under the conditions set forth above were passed by votes of 16 in favor, 0 opposed, with 2 abstentions.

I accept these recommendations with the added stipulation that HV1 hostvector systems be used, as specified in the Genentech request, and an appropriate entry has been made in Appendix E.

In the RAC discussion on the proposed experiments using a SV40 virus vector for cloning the FMD capsid segments in mammalian tissue culture, concern was raised about the potential for recombination between an adventitious picornavirus in the cell culture with the SV40 and FMD viral sequences. The RAC recommended by a vote of 17 in favor, 0 opposed, with 1 abstention, that cloning of FMD capsid protein sequences employing a SV40 deletion vector in a mammalian tissue culture system at the Plum Island laboratories by approved in principle under P3 physical containment. The approval is subject to review by the Working Group of individual experiments.

I accept this recommendation, and an appropriate entry has been made in Appendix E.

VIII. Containment Levels for Recombinant DNA Experiments Involving Neurospora Crassa

Dr. David Perkins of Stanford University proposed that entry 2 in Appendix E be amended to read as follows:

> Unmodified laboratory strains of *Neurospora crassa* can be used in all experiments for which HV1 *N. crassa* systems are approved, provided that only DNA from Class 1 agents is used. For agents other than Class 1, unmodified laboratory strains of *N. crassa* can be used in all experiments for which HV1 *N. crassa* system are approved, provided that these are carried out at physical containment one level higher than required for HV1. However, if P3 containment is specified for HV1 *N. crassa,* this level is considered adequate for unmodified *N. Crassa.* Care must be exercised to prevent aerial dispersal of macroconidia, in accordance with good laboratory practice.
>
> Mutationally modified strains of *N. crassa* specified as HV1 in Appendix D can be used

in all experiments for which HV2 *N. crassa* systems are approved, provided that only DNA from Class 1 agents is used.

In the submission, Dr. Perkins argued that present containment levels cannot be justified by any demonstrated hazard, especially when only Class 1 agents are involved, and that present containment levels impose a serious and unnecessary handicap on genetic analysis of *Neurospora.*

Three letters were received strongly supporting this proposal. One commentator said that it was his experience that contamination by airborne *Neurospora* conidia is not a serious problem. Another commentator stated:

. . . Since *Neurospora crassa* is not a pest or pathogen, there is no justification for imposing more stringent containment conditions for research with Neurospora than for investigations with yeast or animal viruses. The current Guidelines are seriously hindering the use and development of Neurospora as a subject for recombinant DNA research.

The RAC recommended approval of the proposal by a vote of 10 in favor, 0 opposed, with 5 abstentions.

I accept this recommendation, and entry 2 of Appendix E has been modified to reflect this decision.

IX. Request to Employ a Conjugative Plasmid To Transfer Neurospora Crassa DNA

Dr. Norman Giles of the University of Georgia in a letter dated March 18, 1981, requested permission to use a conjugative plasmid to transfer the *qa-2* gene of *Neurospora crassa* among *E. coli* K–12 strains. The *N. crassa qa–2* gene would be ligated into a derivative (pVK57) of the mobilized plasmid RSF2124.

In support of his proposal, Dr. Giles argued that (1) no *Neurospora* protein other than the *qa-2* protein (catabolic dehydroquinase) is produced in *E. coli* transformed with plasmid pVK57; no hybrid or non-functional proteins are produced, and (2) the catabolic dehydroquinase synthesized by *Neurospora* is identical to that synthesized by *E. coli,* based on heatstability, sedimentation, and amino acid composition. In addition, fragments close to the NH_2-terminal end have been sequenced and are identical in *E. coli* and *Neurospora crassa.*

A **Federal Register** announcement of Dr. Gilles' request appeared on March 20, 1981 (46 FR 17997). During the thirty day comment period, no comments were received.

The RAC discussed the request at its April 23–24, 1981 meeting. Noting that the *qa-2* fragment was relatively well-defined, RAC recommended approval of the proposal by a vote of 17 in favor, 0 opposed, with no absentions. P2 containment conditions were specified.

I accept this recommendation. A new entry, number 33, has been added to Appendix E as follows:

A conjugative plasmid may be used to transfer among *E. coli* K–12 strains at P2 physical containment the qa-2 gene of *Neurospora crassa* ligated to a mobilizable plasmid.

X. Request To Use An E. Coli Strain Containing Mu Phage Insertions

Dr. Darold Holten of the University of California at Riverside, in a letter dated March 18, 1981, requested permission to utilize the *E. coli* strain DF214 (or derivatives thereof), and plasmid vectors (e.g., pBR322, pBR325) to clone rat cDNA. Strain DF214, a K–12 derivative,contains (1) a Mu phage insertion in the phosphoglucose isomerase gene, and (2) a Mu lysogen in an unknown location. Dr. Holten calculates the frequency of Mu lysis and transduction in DF214 to be of the order of 10^{-12} to 10^{-14}.

The **Federal Register** of March 20, 1981 (46 FR 17997) carried an announcement of Dr. Holten's proposal. No comments were received during the thirty day comment period.

The RAC discussed the proposal at the April 23–24, 1981 meeting and recommended by a vote of 17 in favor, 0 opposed, with no absentions, that the initial screening of the rat library be done at P2 containment; after the clone of interest has been purified, it may be worked with at P1 containment.

I accept this recommendation, and a new entry, item 34, will be added to Appendix E as follows:

E. coli K–12 strain DF214 (or derivatives thereof) and plasmid vectors (e.g., pBR322, pBR325) may be used to clone rat cDNA under P2 conditions. After the clone of interest has been purified, it may be worked with under P1 containment.

Additional Announcements of The Director, NIH

Section IV–E–1–b–(3)–(d) of the Guidelines gives responsibility to the Director, NIH, for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

Accordingly, several requests for authorization to culture, on a large-scale, recombinant DNA host-vector systems have been received and reviewed by the NIH.

I. Professor Barry T. Nall

On June 16, 1981, the Director, NIH, on the recommendation of the RAC, approved a request from Professor Barry T. Nall, University of Texas Health Science Center at Houston, Houston, Texas 77025, for the large-scale culture of EK1 host-vector systems into which have been ligated recombinant DNA plasmids containing *E. coli* DNA and *Saccharomyces cerevisiae* genes for cytochrome *c.*

The principal investigator is Dr. Nall. The work is to be done at the P1–LS level.

III. Cetus Corporation

On June 16, 1981, the Director, NIH, on the recommendation of the RAC, approved requests from Cetus Corporation, 600 Bancroft Way, Berkeley, California 94710, for large-scale culture of Ek1 *E. coli* or NV1 *Bacillus subtilis* containing plasmids coding for human beta-1 fibroblast interferon.

The request was approved with the understanding that Cetus Corporation has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigators or Drs. Michael W. Conrad and Wolfgang H. Hanisch. The work is to be done at the P1–LS level.

III. Johns Hopkins University

On June 16, 1981, the Director, NIH, on the recommendation of the RAC, approved a request from Professor Hamilton O. Smith, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, for the large-scale culture of *E. coli* containing recombinant plasmids comprised of *E. coli* and *Haemophilus haemolyticus* DNA; the *Haemophilus* DNA consists of two of the genes that constitute the *Hha*II restriction/modification system.

Professor Smith is the principal investigator. The work is to be done at the P1–LS level.

Dated: June 22, 1981.

Donald S. Fredrickson, M.D.,

Director, National Institutes of Health.

Note.—OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.* Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only vitually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has

been determined to be not cost effective or in the public interest to attemp to list these program. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8–(b)–(4) and (5) of that Circular.

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